DOTS
a platform by sbi

CGQ user guide

A handbook for cell density monitoring in shake flask and bioreactor applications

Revision 2

sbi SCIENTIFIC BIOPROCESSING

Table of contents

Introduction and general considerations

Welcome to the CGQ user guide and congratulations for your decision of choosing the DOTS platform and CGQ system to monitor your cell cultivation applications. The idea of this user guide is to give you access to all information required to install and use the CGQ within your laboratory everyday life. Generally, it is strongly recommended to read this user guide prior to any installation or operation works with the CGQ system.

Important aspect:

This kind of grey box will be used throughout the complete document for the indication of important aspects, hints, or summaries.

Caveats are indicated by yellow warning signs.

Dangers and risks are indicated by red danger signs.

To ensure that this user guide provides all information you need during your work with the CGQ, we at SBI are reliant on your feedback. Do not hesitate to contact us to share your ideas regarding errors, missing information, or incomprehensibilities so that we can improve this document and keep it up to date with your requirements.

This user guide is under continuous development and should be recognized as preliminary. This user guide, the DOTS platform and the CGQ devices may be subject to changes and improvements without further notice. In case of any question that may arise during the work with CGQ and DOTS Software, do not hesitate to contact us (Contact details on pp. 48).

CGQ user guide revisions:

Revision 0	02.09.2022	Initial document release
Revision 1	19.04.2023	Updated illustration of BioR LEDs
Revision 2	21.11.2023	Updated SBI North America address

The CGQ system – an overview

The CGQ is an analytical laboratory device that is primarily intended to be used for noninvasive cell density monitoring in real-time, especially in shake flasks (CGQ) and bioreactor (CGQ BioR) applications. However, even completely different applications requiring the real-time analysis of particle concentrations can be addressed by the CGQ system.

Figure 1: The CGQ system – Cell density monitoring for shake flasks and bioreactors.

The CGQ system is part of the DOTS platform and consists of three different main components, the CGQ Sensor, the CGQ Hub and the DOTS Software. The CGQ Sensor is the physical heart of the CGQ-system, performing the measurements and subsequent initial data processing tasks. Several CGQ Sensors are connected to the CGQ Hub, which organizes all the communication between the DOTS Software and the CGQ Sensor. Furthermore, the CGQ Hub acts as the central power supply for all the connected CGQ Sensors. Finally, the DOTS Software is the user's window to the world of high precision real-time analysis of cell densities in a broad variety of cultivations processes and applications. DOTS Software performs visualization, data analysis, post processing, exporting and controlling, to provide CGQ users with an intuitive solution to watch, analyze and store their experimental data. This user guide contains all information on the CGQ hardware. The control of CGQ hardware by DOTS Software is documented in the DOTS Software User Guide.

Absolute maximum ratings

Power supply input

voltage	90 – 264 VAC (47 – 63 Hz)
current	1 – 2 A

Power supply output

voltage	5 VDC
current	6 A

Power supply

operating temperature	-30 – 60 °C

CGQ Hub input

voltage / ripple	5.0 VDC / 0.1 VDC

CGQ Hub output

voltage	5.5 VDC
current	3 A

CGQ operating temperature 10 – 50 (75)[1] °C

1 The CGQ has been operated for weeks at 75°C without any noticeable damaging; however there is currently not enough experience with such high temperatures to specify them as typical operation condition. While it is not guaranteed to work at 75°C, the CGQ will do that in most expectable cases. Operating the CGQ above 50°C requires special darkening covers, which can withstand elevated temperatures and which are not part of the standard device.

Recommended operating conditions

Temperature	10 – 50 °C (Ensure to let the CGQ Sensors adjust to the operating temperature for 30 min.)
Humidity (relative)	0 – 80% (non-condensing)
Shake flask filling volume	
optimal range	10 – 15%
good range[1]	5 – 25%
applicable range[2]	2 – 30%
extended range[3]	0 – 50%
Shaking speed	
optimal range[4] using screws	150 – 350 rpm
shaking diameter ≤ 2.5 cm	0 – 350 rpm
shaking diameter ≤ 5.0 cm	0 – 250 rpm
using sticky pads[5]	0 – 200 rpm

1 Measurement quality should be as good as for the optimal range, in few cases slightly reduced precision or weak artifacts might be observed.

2 Measurement quality should be acceptable, in some cases reduced precision or artifacts might be observed.

3 Measurement quality can be acceptable, in many cases reduced precision or artifacts might be observed, filling volumes above 50% shouldn't be used to avoid spilling of the liquid during shaking.

4 Use these speeds for optimal measurement results, for other shaking speeds within the general specification range, in few cases slightly reduced precision or weak artifacts might be observed.

5 To ensure safe shaking condition, always refer to the user guides of your sticky pad manufacturer, if recommended shaking speed limits in the sticky pad user guides are lower than those denoted here, use only those shaking speeds specified in the sticky pad user guides

Ambient light[1]

optimal CGQ performance[2]	
- with shake flasks[3]	coverless
- with bioreactors[4]	coverless
- phototrophic cultivations[5]	coverless

1 The CGQ actively compensates ambient light. Depending on the application specific ambient light and cultivation conditions, this compensation may be incomplete. Constant ambient light can be compensated efficiently by the CGQ. Strong changes of the ambient light intensity (e.g. from absolute darkness at night to bright daylight at a sunny mounting position) may be visible as step-like artifacts in the measurement data.

The susceptibility for ambient light induced measurement artifacts may be increased for ambient light transitions, which include periods of absolute darkness with pronounced artifacts especially in shaken cultivation systems.

2 Optimal measurement performance with regard to sensitivity at low cell densities, optimal signal-to-noise ratio and minimized number and size of measurement artifacts.

3 In accordance with the above remarks, shake flask cultivations should be performed with CGQ covers, when operated under ambient light conditions with periods of absolute darkness.

4 In accordance with the above remarks, bioreactors as non-shaken systems are less susceptible to strong changes of environmental light intensities.

5 In accordance with the above remarks, phototrophic cultivations provide constantly defined ambient light conditions, which can be efficiently compensated by the CGQ. The limit of ambient light compensation is the CGQ's saturation intensity, which may be reached under certain phototrophic lighting conditions.

Warnings

Do not use the CGQ system or any of its components in water bath shakers! This might result in electric shocks, which could damage your health, the CGQ and any other electric device around.

Do not look into the beam of any of the CGQ Sensor LEDs! Their emitted light is of high intensity and might damage your eye or retina. Wear protective eye wear. Avoid direct eye and skin exposure to the CGQ Sensor LEDs! Always keep a safety distance of >1 m to any active CGQ Sensor LED. Always pause or stop running measurements before operating within this safety distance.

CGQ Sensors (e.g. CGQ BioR) may contain IR-LEDs emitting invisible high intensity light. Do not look into any CGQ Sensor LED to avoid the exposure of your eyes or skin to dangerous invisible infrared light. CGQ Sensors carrying IR-LEDs are labeled with the following warning sign:

Do not touch, wet or electrically bridge the CGQ Hub connectors, especially when the CGQ Hub is connected to a power supply! This might result in damage to your health and/or the CGQ device.

Do not connect any CGQ Sensor via its USB connector, while the CGQ Sensor is connected to the CGQ Hub at the same time! This might result in damage to the CGQ and any other device connected to it via USB.

Be careful when mounting CGQ Sensors into spring clamps to avoid injuries such as contusions, cuts, or bruises.

Be careful when handling the shake flask covers. They might have sharp edges that might cause injuries such as cuts.

Always stop the shaker before handling the CGQ device or the shake flasks. For mounting and dismounting purposes, the shaking should be turned off to avoid damage to your health and to the devices and flasks on the shaker.

Do not spill liquids over any of the CGQ components. Especially the CGQ Sensors might get damaged or the windows above the sensors might become dirty, thus negatively influencing the following measurements.

Do not use inorganic or organic acids and bases, organic solvents or detergents to clean the CGQ! Some solvents or detergents might be allowed for cleaning, but you should only use those being mentioned in the user guide.

Any kind of opening, manipulating or copying CGQ devices as well as decompiling, reverse-engineering, copying or distributing DOTS software or CGQ firmware components is strictly prohibited in accordance with German and international law and may lead to compensation claims.

SBI sensors, devices and other equipment are not intended for medical or military purposes or any other safety-critical applications. It is strictly prohibited to use SBI sensors, devices and other equipment for applications in humans or for applications where sensors are brought in direct contact with foods, drinks, tissues or other goods that are transferred into humans.

Declarations and certificates

CE conformity

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares under its sole responsibility that all devices and equipment being part of the CGQ system and being manufactured by the aquila biolabs GmbH are in conformity with the Council Directives as described in EN IEC 61000-6-2:2019, EN IEC 61000-6-4:2019, EN 61326-1:2013, EN 55016-2-3:2017 + A1:2019, EN 61000-4-2:2009, EN IEC 61000-4-3:2020-09, EN 61000-4-4:2012, EN 61000-4-5:2014 + A1:2017, EN 61000-4-6:2014

This declaration applies to all products with the following identifiers:

CGQ Sensors:

- CGQ-SP-F Revisions: 1.00, 1.10, 1.21, 1.31
- CGQ-SP-B Revisions: 1.00, 1.10, 1.21, 1.31
- CGQ-SP-C Revisions: 1.00, 1.10, 1.21, 1.31

CGQ Hubs:

- CGQ-BS-8-BV Revisions: 1.00, 1.10
- CGQ-BS-16-BV Revisions: 1.00, 1.10

Technical documentation is maintained at the aquila biolabs GmbH headquarter in Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany.

Date of declaration: 01.09.2022
Name, position of the undersigned: Konrad Herzog, Managing Director Research & Development

WEEE conformity

WEEE-Registration-No.: 61144888

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares compliance of all electronic components of the CGQ system with the Council Directive 2012/19/EU.

Electronic components may contain various hazardous substances that could possibly exhibit negative impacts on your health and the environment. In order to avoid those effects aquila biolabs encourages you to make use of the appropriate local take-back and recycling systems for disposing electrical and electronic equipment. By doing this you are furthermore significantly reducing the pressure on natural resources and thus preserve our planet for subsequent generations.

Components that are affected by this declaration carry the following pictogram:

Date of declaration: 01.09.2022
Name, position of the undersigned: Konrad Herzog, Managing Director Research & Development

FCC compliance

This is a Class A product.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

(1) this device may not cause harmful interference, and

(2) this device must accept any interference received, including interference that may cause undesired operation.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Installation

The CGQ system can be used for online biomass monitoring in shake flasks or bioreactor vessels. The main difference is the sensor type: CGQ Sensor for shake flasks, CGQ BioR for bioreactors. The following section explains the components and installation process for shake flasks. Refer to page 27 for bioreactors.

Installing the CGQ on a shaker

Summary of components and use cases

The main components of the CGQ system for shake flasks are the CGQ Sensors and the CGQ Hub. The CGQ Sensors are mounted into spring clamps. Sensor Adapters are used for flask sizes above 100 ml. The flasks are then installed on top of the CGQ Sensors. The CGQ Hub connects all CGQ Sensors to a PC via USB.

Figure 2: CGQ components for shaken applications – Sensor Adapter, CGQ Sensor, CGQ Hubs, Sticky Mat/Clamp Adapter, Bottle Adapter.

The CGQ Sensors are designed to fit into Infors/Lauber clamps and corresponding shaking trays. Sticky Mat/Clamp Adapters are available for shaking trays with differing distribution of screwing threads, and for shaking trays equipped with sticky mats.

Figure 3: CGQ components assembled for Erlenmeyer flasks with Lauber clamps or Sticky Mat/Clamp Adapters and for bottles with Bottle Adapter.

The CGQ and its standard adapters are designed to be used with Erlenmeyer flasks. In case your experiment requires straight flasks, Bottle Adapters are available. The adapters are designed to secure a straight bottle (e.g., a laboratory bottle or serum bottle) on the tray while holding the CGQ sensor at its side. The side-measurement is required due to the thick flask bottom. The CGQ Hub fits on any kind of shaking tray with screw threads or sticky pad.

The following sections guide you through the installation of all system components step-by-step.

Installation on a shaking tray with screw threads

The CGQ Hub's bottom plate exhibits three elongated holes, enabling its installation on each kind of shaking tray using screws. When installing the CGQ

Hub, just move it over the tray until at least two screwing threads are visible through the elongated CGQ Hub bottom plate holes. Then use appropriate screws that fit your tray's screw threads to fix the CGQ Hub on the tray.

Avoid the usage of countersunk head screws, as they might destroy the CGQ Hub bottom plate when being screwed too tightly.

We recommend using a washer per screw for the CGQ Hub installation. Doing this will reduce the mechanical load on the edges of the elongated holes and might thus increase the stability and lifetime of the CGQ Hub bottom plate.

The CGQ Sensors (standalone or in an adapter, see Figure 2) require certain types of metal clamps to be mounted properly (they are designed to fit into Infors/Lauber clamps). If your shaker is already equipped with appropriate clamps, you can directly go to page 21, where the CGQ Sensor installation is described.

If your shaker has a screwing thread geometry and arrangement that fits the clamps being optionally delivered with CGQ Sensors, you can directly install these clamps according to your usual clamp installation process. Otherwise you have to use the Sticky Mat/Clamp Adapters. First remove the protection foil and position the adapter in a way that at least two tray screwing threads are fully visible through the adapter's elongated holes. Now fix the adapter on the tray using appropriate screws.

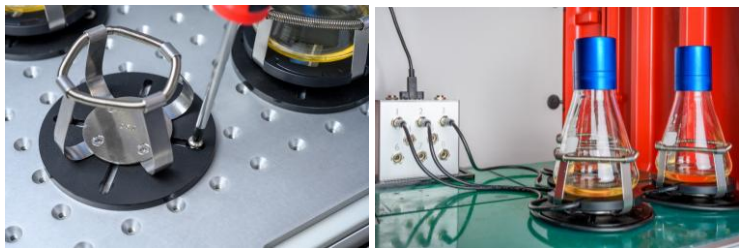


Figure 4: Mounting of Sticky Mat/Clamp Adapter on shaking tray with screwing threads (left) and sticky mat (right).

When using Bottle Adapters, install them just as the Sticky Mat/Clamp Adapters by using at least two screws. Make sure they sit tightly before mounting the flask. The sensor is inserted into the back of the adapter by first opening the lid (remove the screws), place the sensor in the hole and close the lid of the adapter with the screws.

Avoid the usage of countersunk head screws, as they might destroy the adapter when being screwed too tightly.

We recommend using a washer per screw for the clamp mounting adapter installation. Doing this will reduce the mechanical load at the edges of the elongated holes and might thus increase the adapters stability and lifetime.

Installation on a shaking tray with sticky mat

The CGQ Sensors require the use of spring clamps. For the use on sticky mats, Sticky Mat/Clamp Adapters including spring clamps are available (see Figure 2 and Figure 4). These adapters and the CGQ Hub are mounted directly onto the sticky mat.

Remove the protection foil from the CGQ Hub bottom. Place the CGQ Hub on the mat as shown in Figure 4, with the ports of the Hub facing towards the inside of the shaker. Push down the CGQ Hub to make sure that it is in good contact with the sticky mat. At higher shaking frequencies (> 200 rpm), it is recommended to request an alternative bottom plate from SBI, which allows to mount the CGQ Hub on its backside. This may also require an adjusted cable management (Figure 14), make sure to contact SBI before starting your cultivation at high frequencies.

In order to install the CGQ Sticky Mat/Clamp Adapters on a sticky mat, first remove the protection foil from the adapter bottom. Then place the adapter on the mat and push it down to make sure that it is in good contact with the sticky mat. You can pre-mount CGQ Sensors inside the Sticky Mat/Clamp Adapters before placing them in your shaker.

For both, the CGQ Hub and the Sticky Mat/Clamp Adapters, please follow these instructions before starting the shaker to avoid detachment of components during shaking.

Remove dust or any other kind of dirt from components to be installed on the sticky mat, as well as from the sticky mat itself.

The CGQ components can be cleaned using a soft, damp cloth with water. Persistent dirt can be removed using ethanol or isopropanol instead of water. Please wipe away any excess ethanol or isopropanol to avoid chemically induced cracks of the plastic parts. Always ensure that the components are completely dry before powering them. For the sticky mat, refer to your manufacturer's instructions. Usually, sticky mats can be cleaned with water and soft soap. Rinse with clean water afterwards and let air dry completely.

All components to be installed on a sticky mat should be absolutely dry and free of any liquids on the surfaces.

Ensure that all components are fully placed and pressed onto the sticky mats.

After installation of all components check the adhesive strength and proper installation of the components at the desired shaking speed without any shake flasks mounted into the Sticky Mat/Clamp Adapters.

To remove the CGQ from a sticky mat shaker, follow the recommendations of the sticky mat manufacturer. Prior to removal disconnect all cables. Generally, you can remove both, the CGQ Hub and the Sticky Mat/Clamp Adapters by pulling slowly, then wait until the component detaches from the sticky mat.

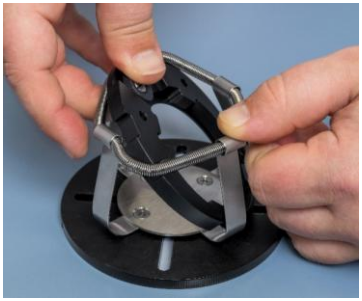
Do not use liquids for the removal of the CGQ Hub from a sticky mat shaker. The liquid might damage the CGQ Hub electronics and, if connected to the power supply, your health.

Mounting a CGQ Sensor

The CGQ Sensors fit into Infors/Lauber spring clamps for 100 ml flasks. For larger flasks, use adapters as shown in Figure 5.

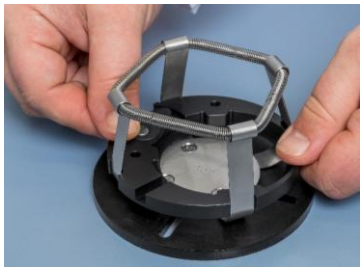
Figure 5: Assembly of CGQ Sensor with Sensor Adapter for 250 ml flask.

Always mount the CGQ Sensor according to the following instructions.



Mount the appropriate adapter size. For flask size 100 ml, the CGQ Sensors can be mounted directly. Align the four cavities on the side with the four metal clips of the clamp.

Figure 6: Mounting the Sensor Adapter in a clamp – Step 1.



Push down until the metal clips snap tightly into the cavities.

Figure 7: Mounting the Sensor Adapter in a clamp – Step 2.

Now insert the CGQ Sensor from above. First pass its cable through the spring clamp, at the spot where you can align the cable with the corresponding cavity in the adapter.

Figure 8: Mounting the CGQ in a Sensor Adapter – Step 1.

Tighten the handle on the adapter to secure the CGQ Sensor in place.

Figure 9: Mounting the CGQ in a Sensor Adapter – Step 2.

Connect every installed CGQ Sensor to a port of your choice on the CGQ Hub. Remember the port, as this will be helpful to identify individual sensors later in the DOTS Software.

Figure 10: Connecting CGQ Sensors to the CGQ Hub.

Be careful when mounting or removing CGQ Sensors into or from spring clamps to avoid injuries such as contusions, cuts or bruises.

It is strongly recommended to bend the single spring clamp clips inwards after mounting the CGQ Sensor. Doing this will improve the shake flask fixation in the clamp, thus preventing unwanted detachment of shake flasks during operation.

Make sure that the CGQ Sensor cable is not pinched between CGQ Sensor and spring clamp. Otherwise, the device might be damaged during shaking.

Before starting a new fermentation at a temperature different to the current CGQ Sensor temperature, ensure that the CGQ Sensors have adjusted their temperature to the desired fermentation temperature. Usually, 30 minutes of temperature adjustment prior to fermentation should be sufficient to avoid temperature induced artifacts in the cell density data.

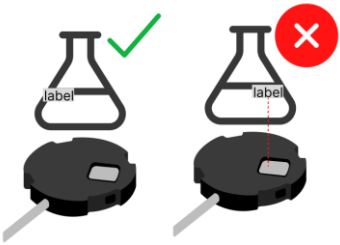
Mounting the shake flask

Before starting a culture, please ensure that the utilized cultivation medium is roughly at the same temperature as the cultivation temperature in the shaker. If a cold liquid is used and put into a warmer shaker, condensation might occur at the flask bottom and over the sensor array. Condensation droplets in the optical path will cause strong measurement artifacts, rendering the cell density measurements useless.

Do not inoculate your flask by throwing a pipette tip or anything similar into the shake flask. For an optimal measurement result, ensure that there are no objects (e.g. pipette tips, feeding capsules, etc.) floating in the liquid in the shake flask.

Prepare your shake flask with cultivation medium and inoculate it.

Insert the flask into the spring clamp as usual.



Turn the shake flask around its vertical axis until neither the white labeling area nor potentially present baffles are located above the sensor array.

Figure 11: Mounting the shake flask on top of the CGQ Sensor.

Cable management within the shaker

Shaking movements create a constant mechanical load for the CGQ USB and power supply cables, which must be minimized by suitable cable routing inside the shaker.

Always mount the CGQ Hub in the shaker, on the tray on which the connected sensors are installed. The CGQ Hub connectors have to face the inner side of the shaker.

Always mount CGQ cables with sufficient play, to reduce the mechanical load of the CGQ Hub connectors.

Always mount and route the CGQ cables inside the shaker in a way that prevents strain on the cables at any time during the shaking movement.

Always ensure that the CGQ USB and power cables are routed with sufficient play before starting the shaking movement.

CGQ USB and power cables should be fixed to the inner wall of the shaker to maintain the state of sufficient play during shaking.

Proper mounting and cable routing within the shaker should be evaluated at low speeds, before starting the intended shaking movement.

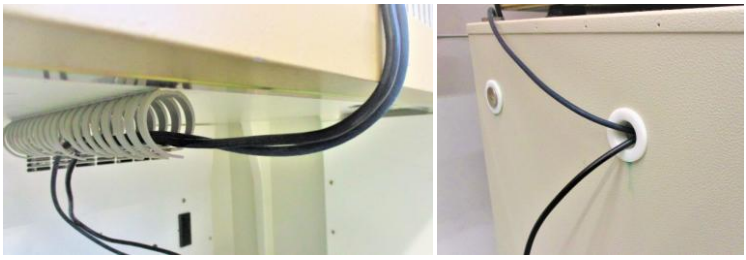


Figure 12: Routing of Power elongation and USB cable.

Power elongation and USB cable should be routed through the front door of your shaker or through side wall openings, as depicted in Figure 12. Use the cable clamp array from your CGQ package as shown in Figure 13 and resize it according to your requirements. It can either be screwed or glued to position.

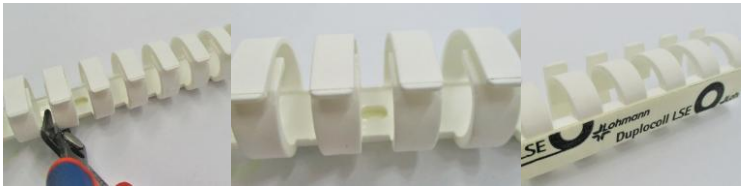


Figure 13: Cable clamp array customization (left), screw mounting hole (center) and sticky back (right).

Use the cable clamp array to guide the cables as depicted below. Both cables must have enough slack between the point where they are held by the clamp and the CGQ Hub. Both cables must be fastened to the CGQ Hub tightly.

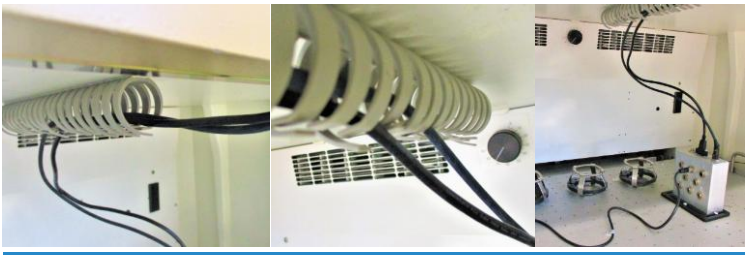


Figure 14: Cable management inside the shaker.

The CGQ Sensor cables should be routed properly on the shaker tray to not hit the wall of the shaker while shaking and should be fixed on the tray using the screwable cable clamps. Ensure that the CGQ Sensor cables have enough slack before connecting them to the CGQ Hub.

Installing the BioR on a bioreactor

The main components of the CGQ system for bioreactors are the BioR Sensor and the CGQ Hub. The BioR Sensor is mounted onto a transparent wall or wall component of a bioreactor vessel. The cell density inside the vessel is measured through the wall. The CGQ Hub connects multiple BioR Sensors to a PC via USB.

Figure 15: The BioR Sensor with elastic foam and Velcro belt.

The CGQ BioR is equipped with a thick layer of elastic foam and with a mounting belt. Both together allow you to install the CGQ BioR at almost any bioreactor in your lab. Initially you need to find a free glass or transparent plastics spot on your bioreactor. Then hold the CGQ BioR with its sensing window onto this transparent spot and fix it there by putting the belt around your bioreactor (or parts of your bioreactor).

Make sure that no probes, labels, marks, scratches or stirrer blades are in front of the sensor window. Assure that the sensor is mounted in a way that there is always liquid in front of it.

Finally ensure that the belt is closed firmly. The sensor cable should point upwards after installation. Exemplary setups are shown below. If the Velcro belt is too short, please use a larger one or combine multiple belts to get a sufficiently long belt for BioR Sensor mounting. In case of mounting problems, do not hesitate to contact us for technical support.

Figure 16: Exemplary setups of BioR Sensors mounted on bioreactors, mounting the belt around the complete bioreactor setup, around the vessel, around the vessel and rods and around two rods.

Do not mount the BioR Sensor on top of marks or labels on the vessel, as this may negatively influence the data quality.

Try to avoid a mounting position, where reflective steel components are directly in front of or in short distance (< 20 mm) to the BioR Sensor.

Always ensure that the BioR Sensor window is completely covered by liquid inside the vessel during your complete process. Foaming and high gas hold-up can significantly interfere with the desired light scattering from growing cells.

The CGQ Hub is placed anywhere near the bioreactor where:

- it is safe from spills of chemicals or culture broth,
- the cable from the BioR Sensor reaches the CGQ Hub,
- the CGQ Hub can be connected to power supply and to a PC running DOTS Software via the supplied USB cable and, if needed, the elongation cable.

Place the CGQ Hub on its flat bottom plate, standing upright. Make sure to fix the CGQ Hub onto a horizontal surface to prevent it from falling over (which might tear on the BioR Sensor cables.

Connect the BioR Sensor to any port of the CGQ Hub. Connect the power cable and the USB cable on top of the CGQ Hub. Take care of proper cable routing.

Connection to DOTS Software

The latest firmware should be installed on CGQ Sensors and BioR Sensors. Contact our support team for instructions on how to update device Firmware.

After having successfully installed the CGQ hardware on your shaker or bioreactor you are now prepared to connect them to DOTS Software. This chapter includes all steps to connect the hardware to DOTS Software. For details on DOTS Software and how to work with it refer to our DOTS Software User Guide.

1. Connect the base station

Connect the power supply elongation cable to the base station and to the power supply.

Connect the power supply to the power supply elongation cable and to the electrical outlet.

Tighten the cap nut of the power supply plug on top of the base station

Connect the USB cable to the base station and to the computer that runs DOTS Software.

Tighten the USB plug screws to fix the USB cable.

As soon as all connections are established, the green LED should start glowing and the CGQ should enumerate as COM device on your computer.

Try to find a good way to guide the cables out of the shaker. The goal must be to reduce the cables' shearing forces during shaking as much as possible, thus ensuring an optimal contact between cable plug and Hub socket. Use the supplies cable management tool (Figure 13) to fix the cables inside the shaker on the shaker wall and then to guide them e.g., through a small slit in the shaker door or through a hole in the shaker wall towards the outside.

Do not insert the screws too far into the USB cable. Stop screwing when the head touches the plastic of the USB cable. Screwing it down into the Hub might break the internal electronics.

2. Mount and connect the sensor plate

Mount the sensor plate into a suitable spring clamp (pp. 21) or at the side wall of your bioreactor (pp. 27).

After mounting the sensor plate, connect it to any port of the base station.

Remember the input number of the connection; this will help you to identify individual sensors later.

3. Prepare your experiment in DOTS Software

Create Objects in DOTS Software and assign your sensors to the Tasks in the Object that use CGQ Sensors.

If you need to identify individual sensors, click on a sensor to open the Device details. Here you see to which port the sensor is connected to, and you can furthermore make the device blink.

3. Mount the shake flask or inoculate your bioreactor

Mount your inoculated shake flask on top of the sensor plate into the spring clamp (pp. 24).

If you are working with a CGQ BioR, inoculate and prepare your bioreactor.

The shake flask should be oriented in a way that neither the white labeling area nor potentially present baffles are located above the sensor array. You can simply rotate the flask until the labeling area is pointing towards the side where the cable is leaving the sensor plate. Wrong positioning might cause measurement artifacts (pp. 41).

Do not mount the CGQ BioR on top of marks or labels on the vessel, as this may negatively influence the data quality.

4. Start your cultivation

All hardware related works have been done right now, so you can close and start your shaker.

If you are working with a CGQ BioR, you can now start your bioreactor.

Start your experiment in DOTS Software.

Cleaning and Disinfection

Ensure that you have disconnected all CGQ devices and cables from any kind of power supply or PC, to prevent damages to the electronics, to connected devices and to your health.

Ensure that all CGQ devices and cables are completely dry after disinfection, before you reconnect them to each other and to the power supply and USB.

Component	Cleaning	Disinfection
CGQ Hub (except for bottom plate)	Gently wipe with (damp) lint-free wipes (e.g., Chemwipes)	Except for the bottom plate wipe gently with 70 % ethanol wipes
CGQ Hub bottom plate	Gently wipe with (damp) lint-free wipes (e.g., Chemwipes)	Special PMMA disinfectants, such as Bacillol® 30 Tissues
Spring clamps	Water and mild soap	wipe gently with 70 % ethanol wipes
Adapters for CGQ Sensors (except for bottom plate)	Water and mild soap	wipe gently with 70 % ethanol wipes
Bottom plate of adapters for CGQ Sensors	Water and mild soap	Special PMMA disinfectants, such as Bacillol® 30 Tissues
CGQ Sensor, BioR Sensor	Wipe of dust from the sensor window with lint-free wipes (e.g., chemwipe). The rest of the sensor plate can be gently wiped with a damp lint-free wipe	The CGQ Sensors CGQ-SP-F, CGQ-SP-B and CGQ-SP-C (see backside label) can be disinfected by wiping them softly with 70% ethanol wipes.

CGQ's basic measurement principles

Optical cell density measurements

The CGQ's technique for noninvasive cell density monitoring in shake flasks is based on the principle of light scattering. Basically, each measurement consists of a sequence of three major steps. (1) Light is irradiated by a LED into the fermentation broth through the transparent vessel wall. While most of the photons go straight through the liquid, some interact with the cells and are scattered towards different directions. (2) The backscattered photons are then detected by a photodiode, which converts the scattered light intensity into a weak electric current. (3) This initial raw signal is subsequently amplified and subjected to various analytical algorithms on the CGQ Sensor, finally yielding the current cell density. The higher the cell density in the fermentation broth, the higher the probability of an incident photon to interact with a cell, so that with increasing cell density more light is scattered towards the photodiode.

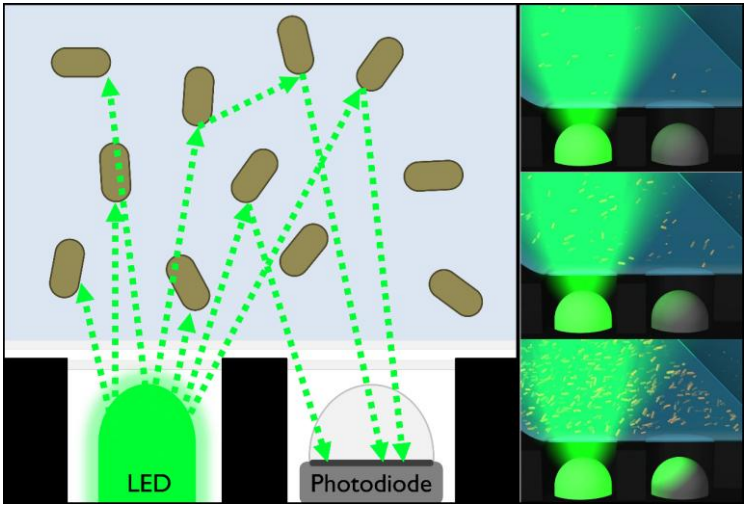


Figure 17: Optical cell density measurement using light scattering.

Each CGQ Sensor comprises highly sensitive sensor technology implemented around an array of LEDs and photodiodes. The CGQ measurements of backscattered light allow for cell density measurements in the range of 0.1 OD_{600} and on the other hand enable you to monitor fermentations that grow beyond 150 OD_{600}. In contrast to typical transmission-based spectrophotometers, the light scattering analysis implemented in the CGQ guarantees cell density measurements over three orders of magnitude without any requirements for diluting or concentrating steps. The CGQ system consequently provides you a simple, efficient, and accurate opportunity to monitor biomass concentrations of your shake flask and bioreactor fermentations noninvasively and without the need for additional devices.

CGQ Sensors are equipped with technology and algorithms to distinguish between ambient light and the backscatter signal, thus allowing for ambient light compensation. This is essential for cell density monitoring in bioreactors, which cannot be covered and darkened as easily as shake flasks. Furthermore, the CGQ is applicable for phototrophic cultivations, as long as the photosynthetic light is not saturating the CGQ sensors. The ambient light compensation is intended for operating conditions with constant or intermediately changing ambient light intensities. Strong intensity changes, especially with periods of absolute darkness, may cause step-like artifacts in the measurement curves. For optimal measurement performance, the use of CGQ covers to darken the shake flasks is still highly recommended. Please also refer to the recommended operating conditions (pp. 9)

While the standard CGQ Sensor peak wavelength is 521 nm, the BioR Sensors contain an additional infrared LED (940 nm). The probability for infrared photons to be scattered by cells is lower than for the standard green light photons, which provides you with a unique opportunity to monitor the growth of high cell density fermentations in the bioreactor with achievable cell densities of 250 OD_{600} (150 g/L) and beyond.

Furthermore, the wavelength of each CGQ Sensor is customizable during the ordering process according to Figure 18. Depending on application specific

requirements, you can choose your preferred wavelength together with our application specialists to avoid an interference of backscatter measurements with secreted or cellular chromophores or fluorophores, such as chlorophyll, heme proteins, GFP, etc.

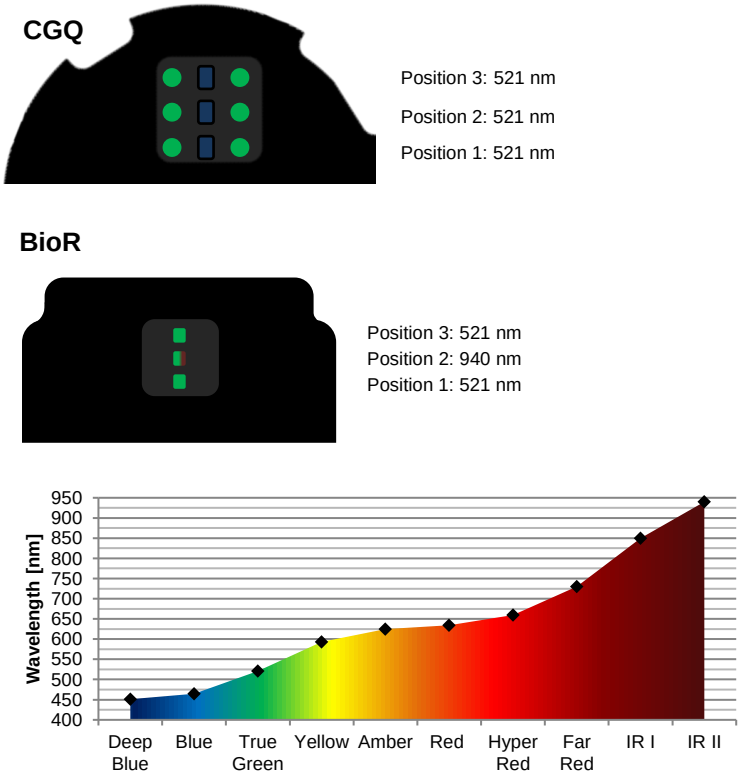


Figure 18: Standard LED colors at the CGQ and BioR measurement positions and LED colors available for wavelength customization of CGQ and BioR Sensors.

Challenges in shaken and stirred vessels

Dynamic and heterogeneous liquid distributions are a key characteristic of shake flask fermentations. Performing highly accurate optical measurements under conditions of continuous shaking is therefore not trivial. Especially the huge variance of observable liquid distributions (for examples see Figure 19), which depend on a variety of different factors (e.g. flask size, flask shape, liquid volume, shaking frequency, shaking radius, liquid viscosity, temperature, etc.), requires adaptive sensor systems and measuring methods. The CGQ combines these two aspects with extensive data analysis to allow cell density measurements under almost every imaginable shaking condition. Using an array of LEDs and photodiodes, each CGQ Sensor can adaptively measure the backscattering intensity at different radial positions, thus accounting for a broad variety of liquid distributions in shake flask fermentations.

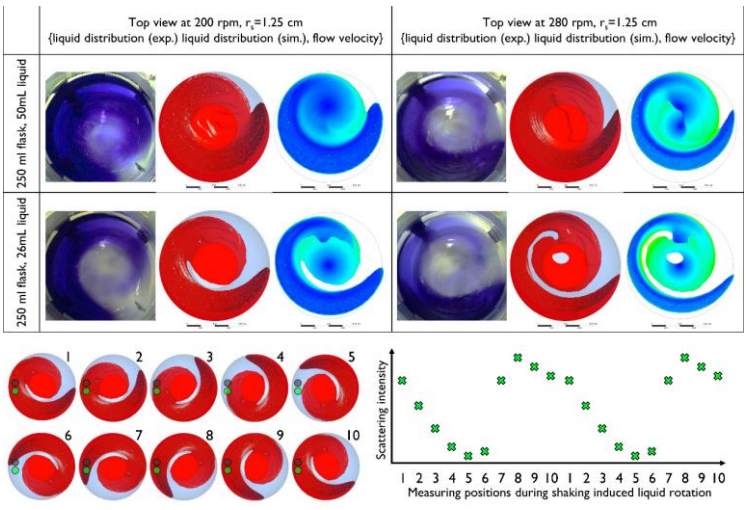


Figure 19: Liquid distribution and CGQ's cell density monitoring principle in continuously shaken systems.

The CGQ does not treat the moving-liquid-induced backscattering signal fluctuations as noise, but as a source of valuable information. For determining one cell density value, the CGQ Sensor collects about one million of single backscattering intensities per second; thereby creating a series of data points that combines information regarding cell density and liquid distribution on a microseconds-resolution. This basic CGQ measurement principle is schematically depicted in the lower parts of Figure 19. Intensive data analysis is performed on the resulting raw data series, finally yielding a single cell density value. Featuring ultrafast data collection, adaptive data analysis and measuring positions, the CGQ provides you with the unique opportunity of accurate real-time cell density monitoring in continuously shaken systems.

While dynamic liquid distribution is a negligible issue in stirred tank bioreactors, the BioR Sensor is subjected to other bioreactor specific challenges. Due to active aeration and stirring, bioreactors tend to foam much heavier than shake flasks, thus requiring the addition of antifoam. Both, foam and antifoam act as additional scatterers to the BioR Sensor and need to be taken into consideration during data interpretation. Depending on the aeration rate and stirrer speed, the bubble size and distribution change over time and can create a considerable background noise in BioR Sensor measurements. The increase in fermentation system complexity from shake flask to bioreactor also increases the number of factors that may influence the CGQ backscatter measurements. The implications of these factors on data acquisition and interpretation are discussed in a separate chapter on pp. 41.

Correlation between OD_{600} and the CGQ signal

A typical measure for cell density is the optical density, often referred to as OD_{600} at a measurement wavelength of 600 nm and given by Lambert-Beer's law as

$$c = \frac{\log_{10}\left(\frac{I_0}{I_1}\right)}{\varepsilon_\lambda \cdot d} \qquad \{1\}$$

with I_0 the incident light intensity, I_1 the transmitted light intensity, ε_λ the extinction coefficient at a given wavelength λ, c the concentration of the substance to be measured and d the transmitted path length. As widely known, Lambert-Beer's-law holds true only for homogenous dilute solutions, so that concentrations above 0.5 OD_{600} need to be diluted prior to the transmission measurements and scaled accordingly after the measurement. The background of this range limitation is an increasing contribution of other optical effects, especially of multiple scattering, to the transmissivity, which is not described by Lambert-Beer's law.

As described above, the CGQ measures backscattered light intensities. While scattering signals can be used to measure particle concentrations over several orders of magnitude, it must be noticed that there is no physical law to describe the correlation between cell density (OD_{600}) and scattering (CGQ signals) as simple as done for the transmission measurements with the Lambert-Beer law. Nevertheless, it is possible to describe this correlation using more elaborate empirical models to account for effects such as multiple scattering.

At higher concentrations (usually above an OD_{600} of 10) the CGQ signal exhibits a linear correlation with the actual cell density in the shake flask. Lower concentrations (usually below an OD_{600} of 5) give a CGQ signal that increases exponentially with increasing cell density. Between these two zones there is a transition zone, where the exponential correlation changes into a linear correlation.

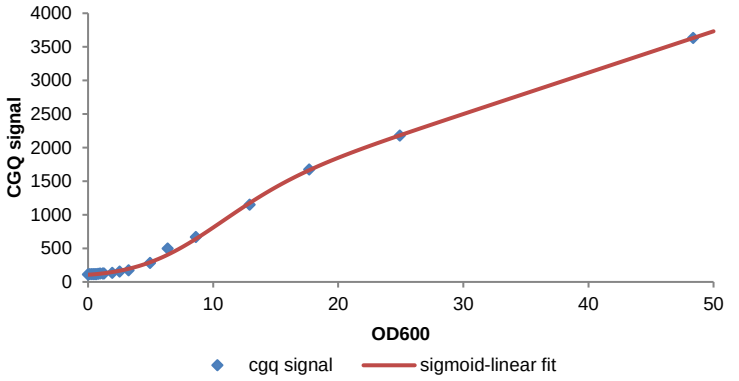


Figure 20: CGQ signal vs. OD_{600} for S. cerevisiae in YPD medium.

As exemplarily shown in Figure 20, this correlation can be described mathematically as a combination of an exponentially sigmoid and a linear function. The DOTS Software implements methods to correlate offline cell density data such as OD or Cell Dry Weight with the CGQ's backscattered light signal. Additional information is provided in the DOTS Software User Guide.

Parameters that influence the CGQ measurements

Each CGQ Sensor is an optical measuring platform designed to provide highest sensitivity and precision in the detection of scattered light, even at lowest particle concentrations. However, this sensitivity means that also changes in the optical environment will be detected by the CGQ. With the ambient light compensating CGQ and BioR Sensors it is possible to monitor growth without the use of covers. To provide a reproducible optical environment for optimal measurements in shake flask applications, it is sometimes recommended to use the shake flask covers, as they create an absolutely constant ambient light zone above the CGQ Sensors, comparable to the dark measurement chamber of a spectrophotometer.

The general comparability of different CGQ Sensors is ensured by factory calibration of the sensor arrays. Comparability of factory calibrated CGQ Sensors means that with an identical scatterer positioned above the sensor array the CGQ signal maximally differs by 5% (typically 1-2%) between two CGQ Sensors.

When comparing the real operating conditions of the CGQ system with those of a typical spectrophotometer, some important differences should be noted.

Shake flasks and bioreactors are not a cuvette!
While cuvettes typically exhibit polished and perfect surfaces, a shake flask bottom or bioreactor side wall usually contains refraction anomalies from the production process and might be furthermore scratched to different extends. Such anomalies or scratches in the transparent vessel wall are not generally disturbing the CGQ measurements. However, they might be an additional source of scattered light, influencing the comparability within a single and between several cultivations.

1. Within a single cultivation there is generally no influence on the data quality as long as the sensor-to-vessel position or orientation is not changed. As soon as the flask or sensor is removed and remounted in a different position/orientation, there might be a different set of

anomalies or scratches in front of the sensors, which might cause jumps in the cell density curve.

Try to avoid removing and remounting of the shake flask or CGQ Sensor during cultivation. If this cannot be avoided, try to remount the system always in the same position/orientation as before to avoid jumps in the cell density curve.

2. Between several cultivations, it usually appears that different flasks are used. As each flask is individual regarding the number, size and distribution of anomalies or scratches, the scattering intensities at equal cell densities differ between these flasks. An exemplary distribution of such differences is shown in Figure 21 for a set of 24 similar shake flasks, which were all measured on the same CGQ Sensor.

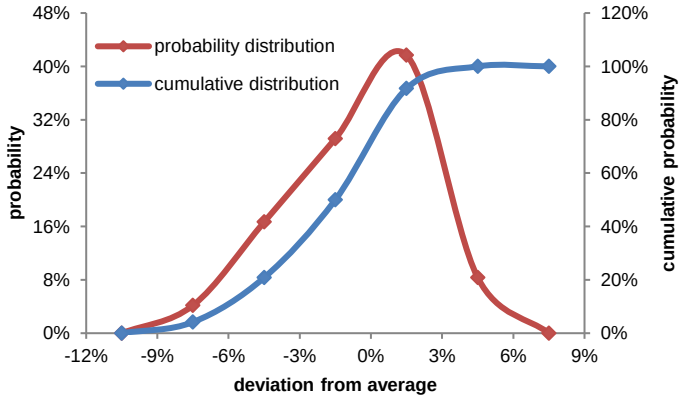


Figure 21: Exemplary CGQ signal differences of a set of 24 shake flasks (aluminum cap, 250 ml), measured at OD_{600} = 4.2, 250 rpm, 25mm shaking diameter, 25 ml filling volume.

As long as the flasks' positions or orientations are not changed during the cultivation, these individual flask differences will (in most cases) only cause offsets in the CGQ signal, which can be removed using the

auto offset feature in DOTS Software (Graph configuration, refer to the DOTS Software User Guide). Flask position/orientation changes during cultivation have been described above.

If a high comparability between several shake flasks is required, try to use flasks with similar degrees and distributions of scratching. In some cases, it may be favorable to preselect a set of highly similar flask, based on measuring their scattering signal on the same CGQ Sensor, at the same cell density and at equal shaking conditions.

Shake flasks and bioreactors are not a blank cuvette!

While cuvettes typically exhibit blank surfaces, shake flasks and bioreactors are usually covered by volume markings, engravings and labeling areas. Even if inscriptions are not located directly in front of the sensor window, they can act as an additional source of scattering if located in the sensor array's field of view. This scattering might interfere with the desired scattering of cells in the fermentation broth and may cause artifacts in the cell density curves.

Always ensure that the CGQ or BioR Sensor is positioned and oriented in a way that no markings, labeling areas or engravings are located in the sensor array's field of view.

Shake flasks and bioreactors are not an even blank cuvette!

While cuvettes typically exhibit even surfaces, shake flasks and bioreactors can be equipped with baffles, emanating either from the side walls or from the bottom of the vessel. Baffles have a curvy geometry, which might act as an additional source of scattering or as a lens if located in front of the sensor array. This scattering might interfere with the desired scattering of cells in the fermentation broth, thus causing artifacts in the cell density curves. Furthermore, it should be noted that remounting the flask during a running cultivation might result in jumps in the cell density curve, similarly as described above for the scratches. Calibration data, if required, should always be collected per flask type.

Always ensure the CGQ or BioR Sensor to be positioned and oriented in a way that no baffles are located in front of the sensor array. If the vessel and baffle geometry permits such an orientation, then try to find a position/orientation so that the outer parts of the sensor array are not covered by the baffles.

Shake flasks and bioreactors are not an even blank quartz cuvette!

While precision cuvettes are typically made of quartz or high-quality glass, a shake flask or bioreactor vessel can be made of glass or plastics, being clear, turbid or colored, single- or double-walled. Generally, this is not problematic for the cell density measurement; however different materials might have different effects on the CGQ signal. As long as the material is at least partially transparent for visible light, the CGQ measurement principle will work, but care must be taken about some limitations.

1. Turbid materials reduce the amount of light going into the flask from the LEDs and leaving the flask or vessel towards the photodiodes, which results in reduced sensitivity in the range of lower cell densities, depending on the degree of turbidity. Turbid materials furthermore act as a strong additional scattering source, thus causing a much stronger background signal.
2. Colored materials might reduce the transmission of light into and out of the shake flask or vessel, similarly to turbid materials. However, the transmissivity of transparent colored materials is usually much higher than that of turbid materials, so that the sensitivity reduction is not as strong as for turbid materials. Nevertheless, applying colored materials or cultivation media with strong absorption in the range of 500 – 550 nm might be difficult, and a LED customization should be considered (pp. 36).
3. Some typical plastics being used in single use flasks exhibit anisotropic structures on a molecular level, which might act as additional scattering source, resulting in increased background signal and cell density curve artifacts.

Generally, it is recommended to be careful when interpreting and comparing results from shake flasks or bioreactor vessels made of different materials, as they might exhibit different background signals, sensitivities or artifacts. Background correction can be done in DOTS Software (Graph configuration, refer to the DOTS Software User Guide).

Always be careful when interpreting and comparing non-calibrated results from shake flasks or bioreactor vessels made of different materials, as they might exhibit different background signals, sensitivities or artifacts.

Shake flasks and bioreactors are not an unagitated even blank quartz cuvette! While cuvettes are typically mounted in an unagitated environment, a shake flask is continuously shaken and a bioreactor is continuously stirred during the measurement, resulting in a continuously moving fluid. All fluidic parameters of those agitated systems, such as filling level, flask or vessel size and shape, shaking or stirring speed, aeration rate, flask cap, inline probes or sampling ports, fermentation broth viscosity, etc. might therefor influence the dynamically measured CGQ signal (refer to pp. 37). The CGQ's data analysis algorithms are made and continuously improved (via firmware updates) to eliminate as many of these signal-influencing fluidic parameters as possible. However, you should not assume that the data from a 2000 ml flask, filled with 400 ml of broth and shaken at 150 rpm can be compared directly to a 250 ml flask, filled with 12.5 ml of broth and shaken at 350 rpm. If you want to do direct and especially quantitative comparisons, calibrations are required as described in the DOTS Software User Guide.

Always be careful when interpreting and comparing non-calibrated results from shake flasks or bioreactors with different fluidic parameters (e.g. filling level, size, shape, agitation speed, cap, fermentation broth viscosity, etc.), as they might exhibit different background signals, sensitivities or artifacts.

Always be careful when interpreting and comparing results from shake flasks or bioreactors, which have been subjected to filling volume changes (e.g. by sampling, feeding or induction). As long as the volume changes are smaller than 20% of the initial filling volume, the effects on cell density curves should be negligible, but larger volumetric changes might result in different background signals, sensitivities or artifacts.

Shake flasks and bioreactors are not an unagitated even blank quartz cuvette representing a steady state system!

While cuvettes are typically filled with a steady state solution as a kind of "current culture snapshot", a shake flask or bioreactor under cultivation is a highly dynamic and complex biological system, which changes continuously over time. These changes can include aspects influencing the fermentation broth's scattering behavior, e.g. filamentous growing organisms can change the viscosity, lipid secreting cells can create biphasic systems, strong morphologic cell modifications like sporulation can change the scattering diameter and secretion of colored substances, secretion or generation of aggregates, cell clumping, biofilm formation, etc. can furthermore contribute to the measured scattering signal.

Always be careful when interpreting and comparing results from cultivations, which are subjected to growth associated changes of parameters that influence the fermentation broth's scattering behavior. Those cultures might exhibit different background signals, sensitivities or artifacts, depending on their respective growth behavior.

Shake flasks are not an unagitated even blank quartz cuvette mounted into a static cuvette holder!

While cuvettes are typically mounted into static cuvette holders, shake flask mounting is done with the help of a flexible clamp. This flexibility may lead to different degrees of deformation of clamps, often depending on their age and position in the shaker. Even slight changes in the clamp deformation can introduce considerable differences in the relative positioning of shake flask and CGQ Sensor, thus influencing the effective scattering liquid volume in the sensor's field of view.

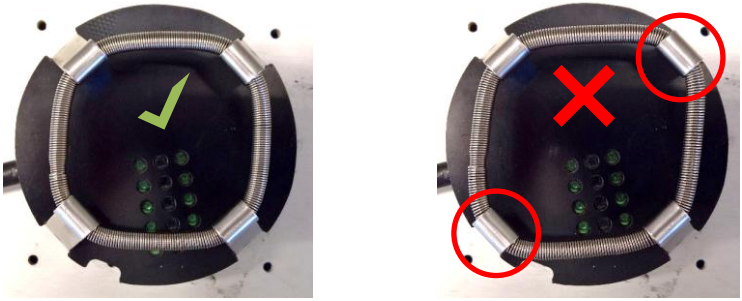


Figure 22: Clamp symmetry and deformation. (left - correct, right - wrong)

Positioning differences due to differently deformed clamps may introduce considerable differences in the CGQ's scattering signal and may therefore negatively influence the flask-to-flask reproducibility and comparability of similar CGQ measurements. In order to avoid this effect, it is strongly recommended to check the symmetry of all clamps each time before mounting a shake flask for CGQ measurements.

Always make sure that clamps are centrosymmetrically aligned and not deformed to avoid flask mispositioning and to improve overall reproducibility.

Contact

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CGQ experiment checklist

- [ ] Is the CGQ Hub connected to the power supply (green LED glowing)?
- [ ] Is the CGQ Hub connected to PC and DOTS Software via USB (CGQ Hub visible and appears as connected in Device list)?
- [ ] Are the CGQ USB and power cables routed with sufficient play within your shaker?
- [ ] Is an available CGQ Sensor connected to the CGQ Hub (Connected sensors are visible in Device list, Devices appear as connected)?
- [ ] Is the shake flask filled with uncooled, noncondensing cultivation medium?
- [ ] Is the medium inoculated without leaving any tips or toothpick inside the flask?
- [ ] Is the shake flask mounted centrosymmetrically above the CGQ Sensor (check clamp deformation symmetry)?
- [ ] Is the shake flask mounted in a way that no labels or labeling fields are located above the CGQ sensor array?
- [ ] Are shake flask and CGQ Sensor correctly covered for applications with increased sensitivity requirements?
- [ ] Is the automated measurement control feature suitable for your shaking conditions (deactivate at <150 rpm)? (This is automatically enabled/disabled if you create your experiment from the respective Quick start Templates)
- [ ] Have you generated, documented and started a new experiment in DOTS Software?
- [ ] Have you added antifoam to your culture in applications with baffled shake flasks?